What is claimed is:

1. A process for the quantitative determination of 25-hydroxy-cholecalciferol in animal feed which comprises the steps of

a) dispersing the feed sample in water and adding to the sample a defined amount of an internal standard compound having a mass different from 25-hydroxycholecalciferol and having a polarity similar to but different from 25-hydroxycholecalciferol;

b) extracting the aqueous dispersion with tert.butyl methyl ether;

c) submitting the ether extract to semipreparative HPLC;

d) collecting the fractions containing 25-hydroxycholecalciferol and the internal standard compound;

e) submitting the fractions collected in d) or an aliquot thereof to HPLC combined with mass spectrometry;

f) determining the MS peak areas of 25-hydroxycholecalciferol and of the internal standard compound added; and

g) calculating the amount of 25-hydroxycholecalciferol by computing the MS peak areas measured.

2. A process as in claim 1 wherein the standard compound is 26,27-hexadeutero-25-hydroxycholecalciferol, 25-hydroxy-ergocalciferol, or 1α-hydroxy-cholecalciferol.

3. A process as in claim 2 wherein the standard compound is 26,27-hexadeutero-25-hydroxycholecalciferol.

4. A process as in any one of claims 1-3 wherein the semipreparative HPLC is carried out on silica gel as the stationary phase and an isopropanol:ethyl acetate:isooctan mixture as the mobile phase.

5. A process as in claim 4 wherein the mobile phase is isopropanol:ethyl acetate:isooctan in a ratio (by volume) of about 1 : 10 : 89.

6. A process as in claim 4 or 5 wherein the stationary phase is Hypersil Si 60, 3 µm.

7. A process as in any one of claims 1-6 wherein the analytical HPLC is carried out in a chromatography system comprising a trapping column on which the substances to be measured are concentrated, and the intrinsic analytical column for separation.

8. A process as in claim 4 wherein the stationary phase in the analytical HPLC is a modified silica gel such as Aquasil C18, 3 µm.

9. A process as in claim 7 or 8 wherein a gradient of water containing 0.05 % (vol/vol) formic acid and methanol containing 0.05 % (vol/vol) formic acid is used as the mobile phase.

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